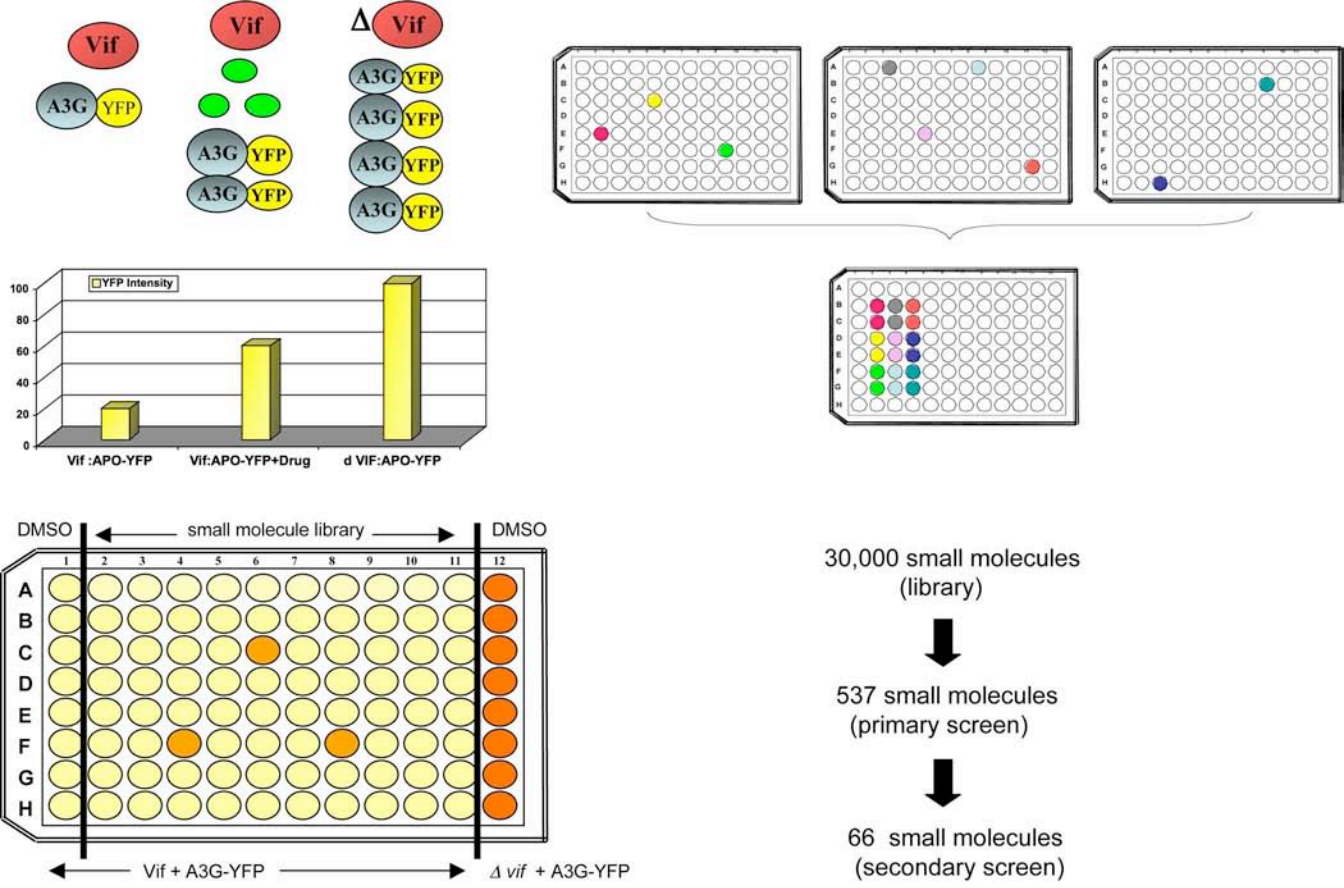
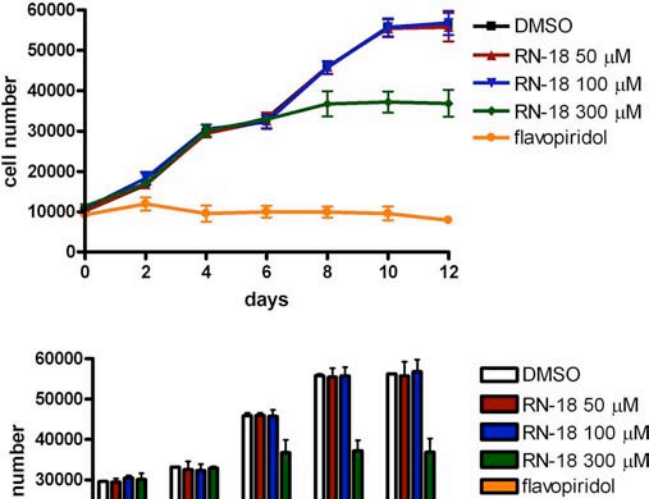
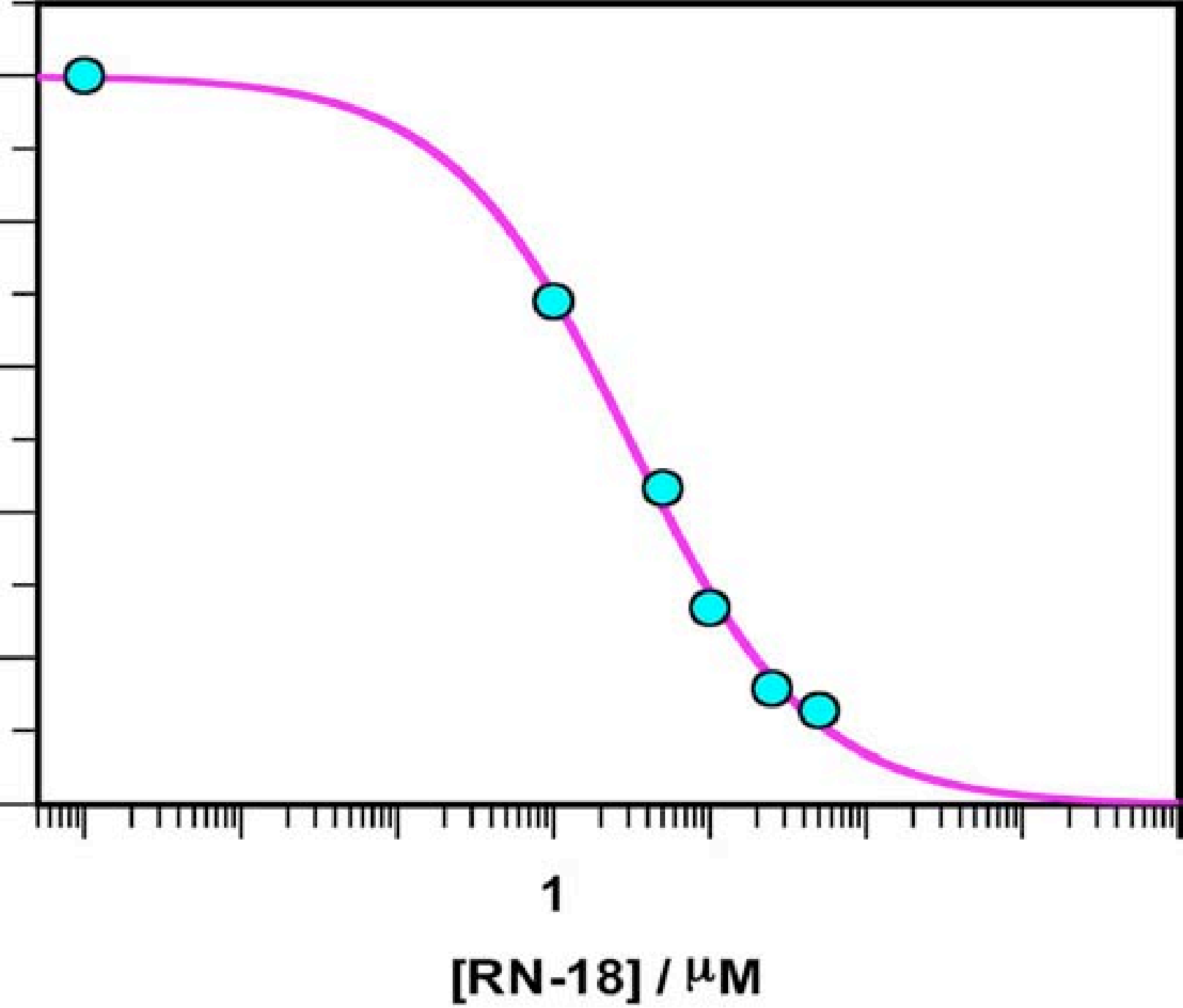


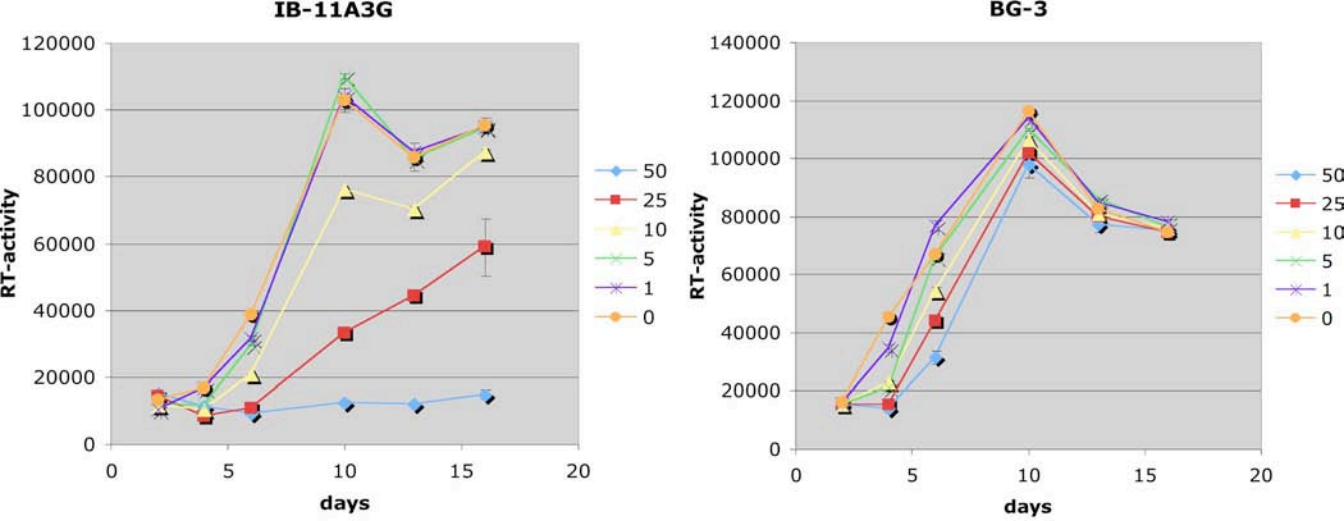
Supplementary Figure 1. Experimental schema for high throughput screening (HTS) to identify HIV-1 Vif inhibitors. (A) Development of fluorescence-based assay to identify Vif inhibitors. 293T cells were co-transfected with YFP-tagged APOBEC3G (A3G) and HIV-1 vectors with (pNL-A1) or without (pNL-A1Δvif) Vif. Cells co-expressing pA3G-YFP and pNL-A1 have lower YFP intensity due to A3G downregulation, and cells co-expressing pA3G-YFP and pNL-A1Δvif have greater YFP intensity. A potential Vif inhibitor (green ovals) would reduce the capacity of Vif to downregulate A3G-YFP, resulting in increased YFP signal intensity. **(B) A typical primary screen plate.** In this plate, wells in column 1 contain cells co-transfected with pNL-A1 and A3G-YFP (in 1% DMSO), and wells in column 12 have cells co-transfected with pNL-A1Δvif and A3G-YFP (in 1% DMSO). The remaining wells contain cells co-transfected with pNL-A1 and A3G-YFP plus 50 μM small molecule per well. More intense YFP signals indicate potential Vif inhibitors. **(C) Secondary screen.** "Hits" from the primary screen were retested in duplicate at 50 μM in a secondary screen. This second screen tested small molecules not only for reproduction of Vif inhibition in the pNL-A1/A3G-YFP assay, but also for false positives as shown by inherent fluorescence, or increased general transfection and/or nonspecific increased protein expression (in an RFP-based assay). **(D) Results of the primary and secondary screens.** Of 30,000 small molecules screened, 537 hits were identified in the primary screen. Of these, 471 were false positives as shown by the secondary screen. "True positives" were the 66 small molecules that passed the secondary screen.



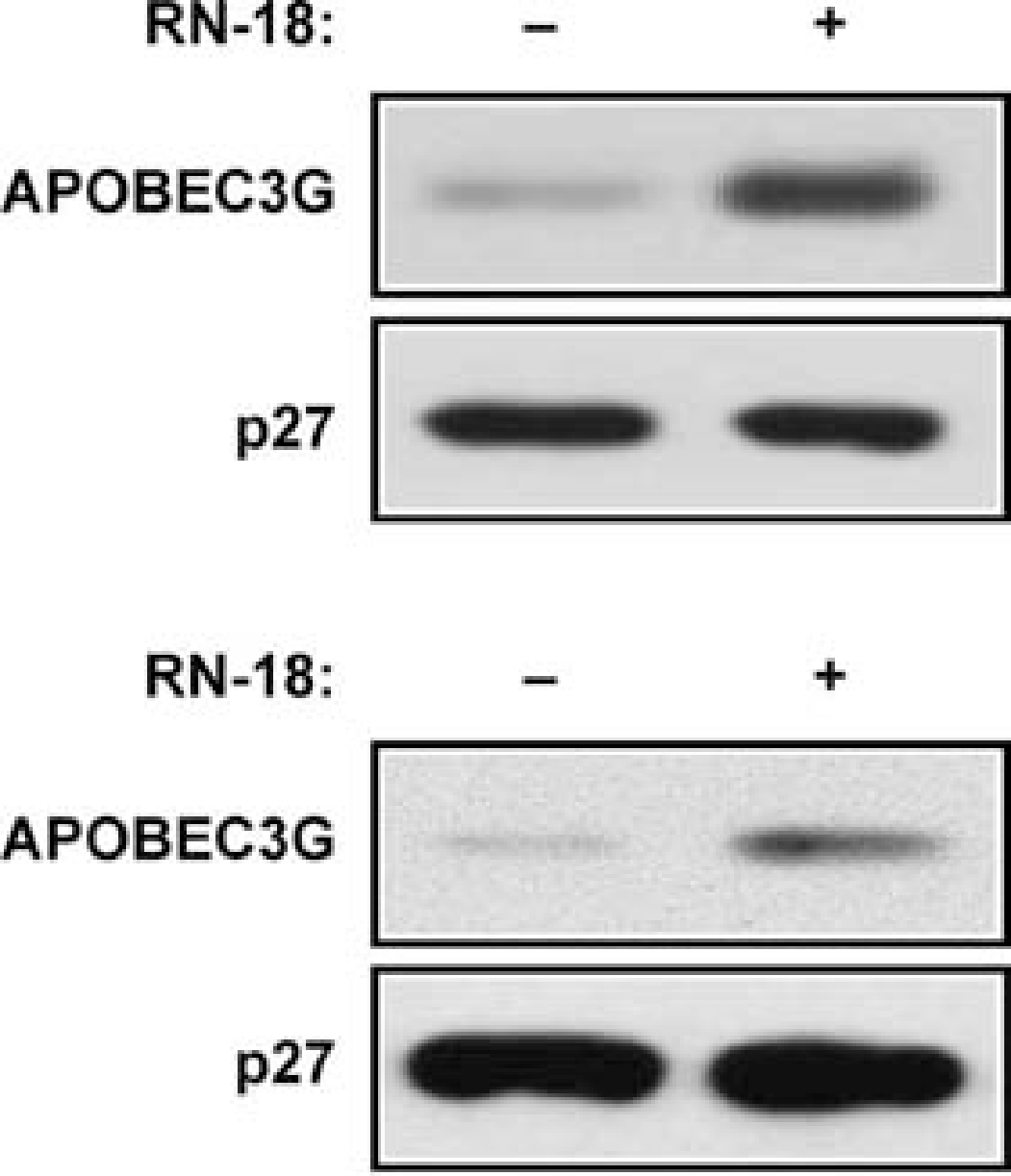
Supplementary Figure 2. The Vif antagonist, RN-18, does not inhibit cell growth nor exhibit toxicity at 50 or 100 μM in H9 cells. (A) Growth curves for H9 cells propagated in triplicate wells and treated every other day with DMSO (negative control) or RN-18 and fresh media. Flavopiridol, which is toxic at high concentrations, 200 nM was used as a positive control. On days 2,4,6,8,10,and 12, cell viability was determined by MTT assay as described in methods. **(B)** Toxicity profiles for RN-18 (or flavopiridol) at days 4-12. H9 cells were treated and processed as specified in (A) only the data is presented in bar graph form.



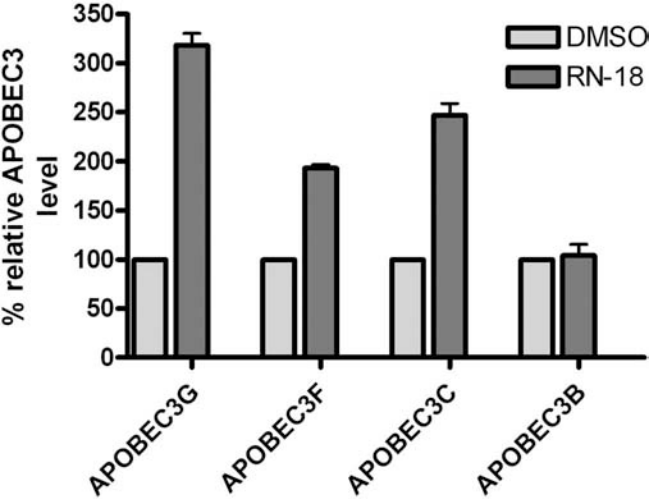
Supplementary Figure 3. RN-18 inhibits HIV-1 replication with an IC50 of ~ 3 μM. Nonpermissive H9 CD+ T-cells were treated with varying concentrations of RN-18, infected with HIV-1, and HIV-1 replication was measured by RT assay. The average % relative infectivity at day 7, near the peak of viral infectivity, was determined from 3 separate RT assays. Grafit software was used to fit the curve and to determine the average IC50 value of 3.0 μM.



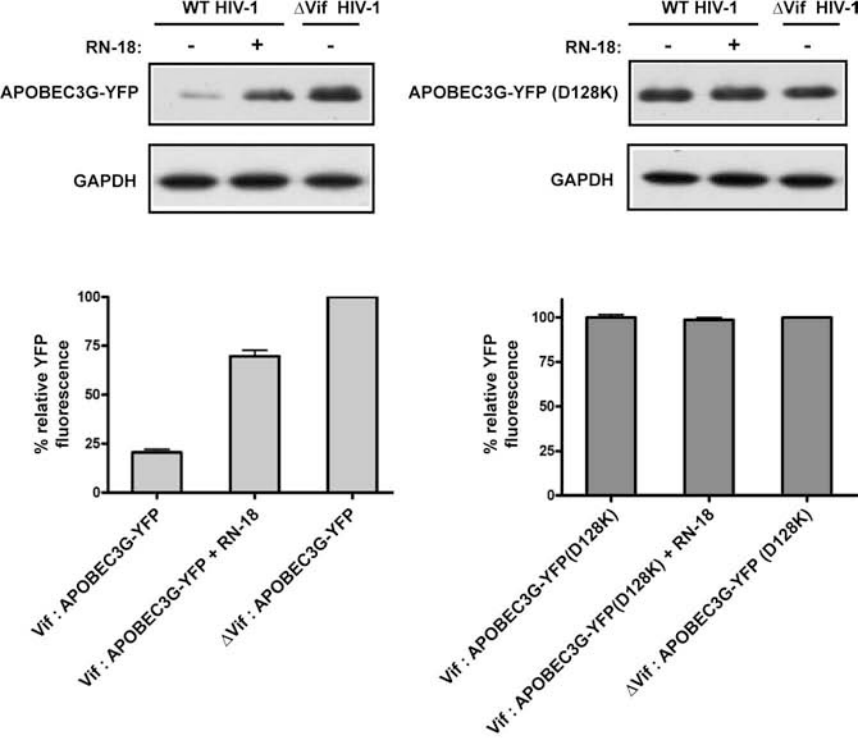
Supplementary Figure 4. The small-molecule Vif antagonist, RN-18, inhibits HIV-1 replication in CEM-SS cells1 expressing APOBEC3G but not in parent CEM-SS cells. **(A)** IB-11A3G cells were infected with HIV-1LAI in the presence of the indicated concentrations of RN-18. Viral replication was monitored at the indicated intervals by RT activity in culture supernatants. **(B)** BG-3 cells were infected and viral replication monitored exactly as in **(A)**.



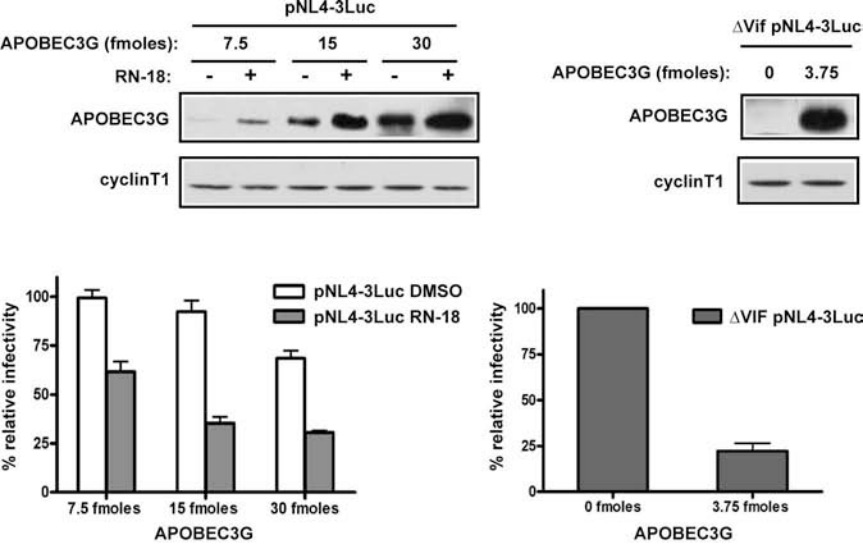
Supplementary Figure 5. The Vif antagonist, RN-18, increases APOBEC3G expression in SIV-1 producer cells and virions. 293 T cells co-expressing SIVmac 239 GFP Δnef and HA-tagged APOBEC3G were cultured for 24 h in the presence (+) or absence (-) of 50 μM RN-18. (A) Total producer cell lysates were analyzed by immunoblotting with antibodies against the HA tag and SIV-1 antigen p27. (B) SIV-1 virions from producer cell supernatants were concentrated, lysed, and analyzed by immunoblotting with antibodies against HA tag and SIV-1 p27.



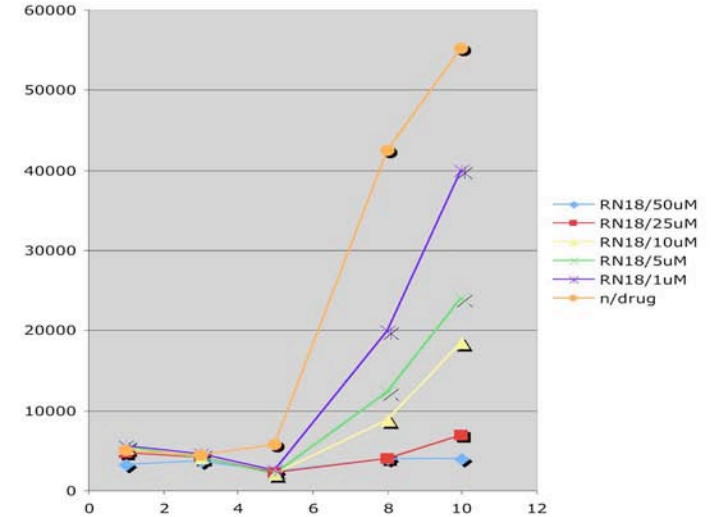
Supplementary Figure 6. The Vif antagonist, RN-18 quantitatively increases expression levels of APOBEC3G, APOBEC3F, and APOBEC3C, but not APOBEC3B in HIV-1 producer cells. 293 T cells co-expressing HIV-1 (pNL4-3LucR-E-) and various HA-tagged APOBEC3 expression vectors were cultured for 24 h in the presence (+) or absence (-) of 50 μM RN-18. Total producer cell lysates from three independent experiments were analyzed by immunoblotting with antibodies against the HA tag and cyclin T1. The APOBEC3 band intensities were quantified using imaging software, and normalized to the internal control, cyclin T1. Data is presented as % relative APOBEC3 level, where samples treated in the absence (-) of 50 μM RN-18 are set to 100%.

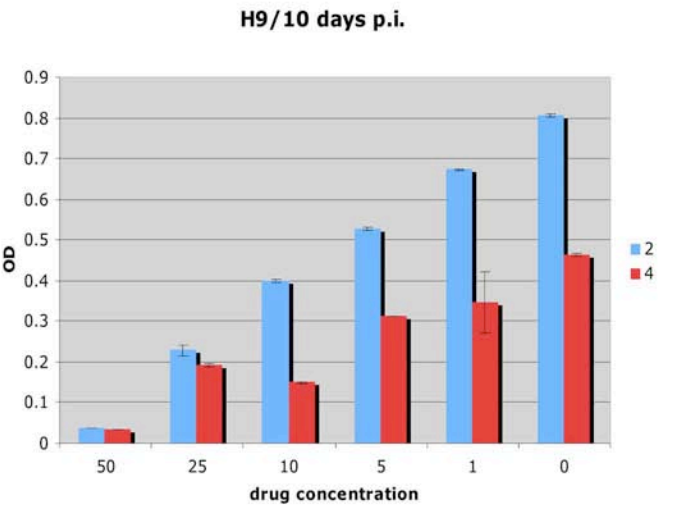


Supplementary Figure 7. The Vif antagonist, RN-18, increases APOBEC3G-YFP expression but not APOBEC3G-YFP (D128K) in HIV-1 producer cells. 293 T cells co-expressing HIV-1 (pNL4-3LucR-E-) and APOBEC3G-YFP or APOBEC3G-YFP (D128K) were cultured for 24 h in the presence (+) or absence (-) of 50 μM RN-18. For a control, 293 T cells co-expressing ΔVif HIV-1 (ΔVif pNL4-3LucR-E) and APOBEC3G-YFP or APOBEC3G-YFP (D128K) were cultured for 24 h in the absence (-) of RN-18. **(A)** Total producer cell lysates were analyzed by immunoblotting with antibodies against the YFP tag for APOBEC3G-YFP or GAPDH. **(B)** Total producer cell lysates were analyzed as in **(A)** only APOBEC3G-YFP (D128K) was detected (along with GAPDH). Lysates from producer cells from three independent experiments were used for quantitative analysis by detection of YFP fluorescence. **(C)** Equal amounts of producer cell lysates were taken in triplicate and YFP fluorescence read and data presented as % relative YFP fluorescence where the ΔVif HIV-1 (with no RN-18 treatment) was set to 100%. **(D)** Producer cell lysates were analyzed, measured, and plotted as in **(C)** only YFP from APOBEC3G-YFP (D128K) was used.

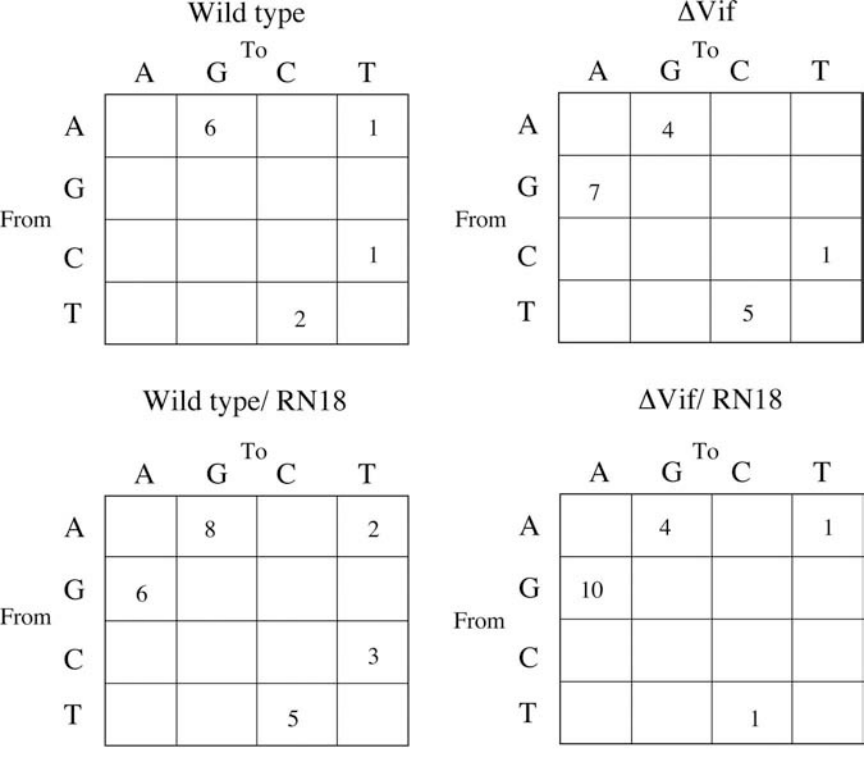


Supplementary Figure 8. The Vif antagonist, RN-18, enhanced APOBEC3G expression in producer cells, resulting in less infectious HIV-1 virions. (A) 293 T cells co-expressing HIV-1 (pNL4-3LucR-E-) and various concentrations of APOBEC3G-HA (3.75, 7.5, 15 fmoles) were cultured for 24 h in the presence (+) or absence (-) of 50 μM RN-18. Total producer cell lysates were analyzed by immunoblotting with antibodies against the HA tag and cyclin T1. **(B)** As a control, 293 T cells were transfected with ΔVif pNL4-3Luc and 3.75 fmoles of APOBEC3G-HA. **(C)** For the infectivity assays, virions were collected from producer cells and all concentrations estimated using p24 ELISA. 293 T cells were infected with 10 ng of HIV-1 virions in triplicate and luciferase signal measured. Data is presented as % relative infectivity, where infectivity without APOBEC3G is set to 100%. **(D)** The % relative infectivity with ΔVif pNL4-3Luc and APOBEC3G is shown as a control, and data presented as previously specified.





Supplementary Figure 9. HIV-1 virions obtained from RN-18-treated cultures are less infectious. H9 cells were infected with HIV-1LAI and maintained in the presence of RN-18 at the indicated concentrations **(A)**. At 10 days post infection, culture supernatants were harvested, normalized to RT activity and used to infect an indicator cell line (MAGI) (PMID:1548759) at two different amounts of input virus (1:2, 1:4 dilution) (error bars=S.D.,n=3) **(B)**.



Supplementary Figure 10. RN18 increases cytidine deamination of endogenous reverse transcription cDNAs. Wildtype or delta-vif virions were produced by transfection of cells stably expressing A3G-YFP and treated or untreated with 150 □M RN18. Individual clones (n=13, 650 nt each) were sequenced to identify virus genome changes.